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     (FILE 'HOME' ENTERED AT 12:51:35 ON 15 AUG 2005)

     INDEX 'ADISCTI, ADISINSIGHT, ADISNEWS, AGRICOLA, ANABSTR, ANTE, AQUALINE,
     AQUASCI, BIOBUSINESS, BIOCOMMERCE, BIOENG, BIOSIS, BIOTECHABS, BIOTECHDS,
     BIOTECHNO, CABA, CANCERLIT, CAPLUS, CEABA-VTB, CEN, CIN, CONFSCI, CROPB,
     CROPU, DDFB, DDFU, DGENE, DISSABS, ...' ENTERED AT 12:51:47 ON 15 AUG 2005
               SEA POLYSIALYLTRANSFERASE
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                 2   FILE AQUASCI
                 2   FILE BIOENG
               128   FILE BIOSIS
                 2   FILE BIOTECHABS
                 2   FILE BIOTECHDS
                66   FILE BIOTECHNO
                 1   FILE CABA
                18   FILE CANCERLIT
               106   FILE CAPLUS
                 4   FILE CONFSCI
                 5   FILE DGENE
                 2   FILE DISSABS
                 1   FILE EMBAL
                87   FILE EMBASE
                82   FILE ESBIOBASE
                 5   FILE FEDRIP
                46   FILE GENBANK
                 4   FILE JICST-EPLUS
                43   FILE LIFESCI
                83   FILE MEDLINE
                 1   FILE OCEAN
                18   FILE PASCAL
                 1   FILE PROMT
               132   FILE SCISEARCH
                38   FILE TOXCENTER
                18   FILE USPATFULL
                 1   FILE WPIDS
                 1   FILE WPINDEX
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     FILE 'SCISEARCH, BIOSIS, CAPLUS, EMBASE, MEDLINE, ESBIOBASE, BIOTECHNO,
     LIFESCI, TOXCENTER, CANCERLIT, PASCAL' ENTERED AT 12:52:55 ON 15 AUG 2005
L2            66 S L1 AND K92
L3             0 S L2 AND SOLUB?
L4             0 S L2 AND RELEASED
L5             0 S L2 AND DETERGENT
L6            18 DUP REM L2 (48 DUPLICATES REMOVED)

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L6 ANSWER 1 OF 18 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation on STN DUPLICATE 1
ACCESSION NUMBER: 2003:377234 SCISEARCH
THE GENUINE ARTICLE: 672HX
TITLE: Functional relationships of the sialyltransferases involved in expression if the polysialic acid capsules of Escherichia coli K1 and **K92** and Neisseria meningitidis groups B or C
AUTHOR: Steenbergen S M; Vimr E R (Reprint)
CORPORATE SOURCE: Univ Illinois, Lab Sialobiol, Div Microbiol & Immunol, Dept Pathobiol, 2001 S Lincoln Ave, Urbana, IL 61802 USA (Reprint); Univ Illinois, Lab Sialobiol, Div Microbiol & Immunol, Dept Pathobiol, Urbana, IL 61802 USA
COUNTRY OF AUTHOR: USA
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, (25 APR 2003) Vol. 278, No. 17, pp. 15349-15359.
ISSN: 0021-9258.
PUBLISHER: AMER SOC BIOCHEMISTRY MOLECULAR BIOLOGY INC, 9650 ROCKVILLE PIKE, BETHESDA, MD 20814-3996 USA.
DOCUMENT TYPE: Article; Journal
LANGUAGE: English
REFERENCE COUNT: 45
ENTRY DATE: Entered STN: 16 May 2003
Last Updated on STN: 16 May 2003

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Polysialic acid (PSA) capsules are cell-associated homopolymers of alpha2,8-, alpha2,9-, or alternating alpha2,8/2,9-linked sialic acid residues that function as essential virulence factors in neuroinvasive diseases caused by certain strains of Escherichia coli and Neisseria meningitidis. SA chains structurally identical to the bacterial alpha2,8-linked capsular polysaccharides are also synthesized by the mammalian central nervous system, where they regulate neuronal function in association with the neural cell adhesion molecule (NCAM). Despite the structural identity between bacterial and NCAM PSAs, the respective **polysialyltransferases** (polySTs) responsible for polymerizing sialyl residues from donor CMP-sialic acid are not homologous glycosyltransferases. To better define the mechanism of capsule biosynthesis, we established the functional interchangeability of bacterial polySTs by complementation of a polymerase-deficient E. coli K1 mutant with the polyST genes from groups B or C N. meningitidis and the control E. coli **K92** polymerase gene. The biochemical and immunochemical results demonstrated that linkage specificity is dictated solely by the source of the polymerase structural gene. To determine the molecular basis for linkage specificity, we created chimeras of the K1 and **K92** polySTs by overlap extension PCR. Exchanging the first 52 N-terminal amino acids of the K1 NeuS with the C terminus of the **K92** homologue did not alter specificity of the resulting chimera, whereas exchanging the first 85 or reciprocally exchanging the first 100 residues did. These results demonstrated that linkage specificity is dependent on residues located between positions 53 and 85 from the N terminus. Site-directed mutagenesis of the **K92** polyST N terminus indicated that no single residue alteration was sufficient to affect specificity, consistent with the proposed function of this domain in orienting the acceptor. The combined results provide the first evidence for residues critical to acceptor binding and elongation in **polysialyltransferase**.

L6 ANSWER 2 OF 18 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN
ACCESSION NUMBER: 2003:530477 BIOSIS
DOCUMENT NUMBER: PREV200300533476
TITLE: Low molecular weight inhibitors of polysialytransferase.

AUTHOR(S): Steenbergen, S. M. [Reprint Author]; Vimr, E. R. [Reprint Author]
CORPORATE SOURCE: University of Illinois, Urbana, IL, USA
SOURCE: Abstracts of the General Meeting of the American Society for Microbiology, (2003) Vol. 103, pp. S-258. http://www.asmusa.org/mtgsrc/generalmeeting.htm. cd-rom.
Meeting Info.: 103rd American Society for Microbiology General Meeting. Washington, DC, USA. May 18-22, 2003. American Society for Microbiology.
ISSN: 1060-2011 (ISSN print).
DOCUMENT TYPE: Conference; (Meeting)
Conference; Abstract; (Meeting Abstract)
LANGUAGE: English
ENTRY DATE: Entered STN: 12 Nov 2003
Last Updated on STN: 12 Nov 2003

AB Polysialic acid (PSA) capsules are critical virulence determinants in sepsis and meningitis caused by Escherichia coli K1 and **K92**, Neisseria meningitidis groups B and C, Moraxella nonliquefaciens, and Pasteurella (Mannheimia) haemolytica type A2. The sialic acid residues in these capsules are synthesized as one of three glycoketosidic chemotypes: (i) alpha2,8, (ii) alpha2,9, or (iii) alternating alpha2,8/2,9 by linkage specific glycosyltransferases called **polysialyltransferase** (polyST). PolySTs catalyze the successive additions of N-acetylneuraminic acid (Neu5Ac) residues from the activated sugar nucleotide donor CMP-Neu5Ac to the nonreducing termini of nascent (acceptor) PSA chains that are finally anchored to the outer membrane through phosphodiester linkages to phosphatidic acid. The alpha2,8 chemotype has resisted all efforts to date to develop safe and effective capsule-based vaccines. As an alternative to vaccinology, we have investigated the structural and functional relationships between different polySTs and here report the effects of low molecular weight donor or acceptor analogues on polyST activity. Free Neu5Ac was neither an inhibitor nor acceptor, while 10 mM cytidine was only slightly inhibitory at this concentration. In contrast, CTP, oxidized (2',3'-dialdehydo) CTP, CDP, and CMP all inhibited polyST, with CMP effective (80% inhibition) at 0.05 mM. These results indicate that polyST preferentially recognizes the donor CMP moiety of CMP-Neu5Ac. Among several polyanionic acceptor analogues tested, only heparin was an inhibitor. The salt-dependency of inhibition and abrogation of inhibition by heparinase pretreatment demonstrated the electrostatic interaction between heparin and polyST. Oxidized compounds with adjacent aldehydes (dialdehydo) are particularly interesting as they are likely to provide site-directed, covalent inhibition that can be used to more precisely identify catalytic polyST residues. Specific polyST inhibitors could have broad utility as inhibitors of certain invasive pathogens.

L6 ANSWER 3 OF 18 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN
ACCESSION NUMBER: 2003:30389 BIOSIS
DOCUMENT NUMBER: PREV200300030389
TITLE: Structural determinants of linkage specificity in the **polysialyltransferase** family.
AUTHOR(S): Steenbergen, Susan M. [Reprint Author]; Vimr, Eric R. [Reprint Author]
CORPORATE SOURCE: Laboratory of Sialobiology, Department of Pathobiology, University of Illinois at Urbana-Champaign, 2001 South Lincoln Avenue, Urbana, IL, 61802, USA
SOURCE: Glycobiology, (October 2002) Vol. 12, No. 10, pp. 704. print.
Meeting Info.: 7th Annual Conference of the Society for Glycobiology. Boston, MA, USA. November 09-12, 2002. Society for Glycobiology.
ISSN: 0959-6658.
DOCUMENT TYPE: Conference; (Meeting)
Conference; (Meeting Poster)

Conference; Abstract; (Meeting Abstract)
LANGUAGE: English
ENTRY DATE: Entered STN: 8 Jan 2003
Last Updated on STN: 8 Jan 2003

L6 ANSWER 4 OF 18 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN
ACCESSION NUMBER: 2003:30388 BIOSIS
DOCUMENT NUMBER: PREV200300030388
TITLE: Initiation and elongation of polymer chains in polysialic acid biosynthesis.
AUTHOR(S): Vionnet, Justine [Reprint Author]; Vann, Willie F. [Reprint Author]
CORPORATE SOURCE: Laboratory of Bacterial Toxins, Center for Biologics Evaluation and Research, Bethesda, MD, 20892, USA
SOURCE: Glycobiology, (October 2002) Vol. 12, No. 10, pp. 703-704. print.
Meeting Info.: 7th Annual Conference of the Society for Glycobiology. Boston, MA, USA. November 09-12, 2002. Society for Glycobiology.
ISSN: 0959-6658.
DOCUMENT TYPE: Conference; (Meeting)
Conference; (Meeting Poster)
Conference; Abstract; (Meeting Abstract)
LANGUAGE: English
ENTRY DATE: Entered STN: 8 Jan 2003
Last Updated on STN: 8 Jan 2003

L6 ANSWER 5 OF 18 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN
ACCESSION NUMBER: 2002:608249 BIOSIS
DOCUMENT NUMBER: PREV200200608249
TITLE: Biosynthesis of the polysialic acid virulence factor.
AUTHOR(S): Steenbergen, S. M. [Reprint author]; Vimr, E. R. [Reprint author]
CORPORATE SOURCE: University of Illinois, Urbana, IL, USA
SOURCE: Abstracts of the General Meeting of the American Society for Microbiology, (2002) Vol. 102, pp. 287-288. print.
Meeting Info.: 102nd General Meeting of the American Society for Microbiology. Salt Lake City, UT, USA. May 19-23, 2002. American Society for Microbiology.
ISSN: 1060-2011.
DOCUMENT TYPE: Conference; (Meeting)
Conference; Abstract; (Meeting Abstract)
LANGUAGE: English
ENTRY DATE: Entered STN: 27 Nov 2002
Last Updated on STN: 27 Nov 2002

AB Escherichia coli K1, **K92** and groups B or C Neisseria meningitidis synthesize polysialic acid (PSA) capsules as the primary virulence factors in these pathogens, and are the targets of extensive vaccine development efforts. PSAs, lipid-linked homopolymers of over 100 sialic acid residues, are synthesized by linkage specific glycosyltransferases called **polysialyltransferases** (polySTs), which catalyze the successive addition of sialyl residues from the activated sugar nucleotide donor CMP-sialic acid to the non-reducing ends of nascent PSA chains. How the biosynthesis of these polymers is initiated or terminated is unknown, as is the molecular basis for elongation, especially how linkage specificity is controlled. The primary structures of the E. coli K1 polyST (synthesizes alpha2,8-PSA) and E. coli **K92** polyST (synthesizes repeating alpha2,8/2,9-PSA) are 82.6% identical, indicating that a relatively small number of amino acid differences account for linkage specificity. On the basis of these primary structural differences, we constructed chimeras of the K1 and **K92** enzymes by overlap extension PCR and characterized the resulting PSA products in a novel in vivo complementation system. This

system is an E. coli K1/K-12 hybrid engineered to express a functional capsular polysaccharide translocation and assembly system, but with defective endogenous polyST and sialic acid aldolase activities. Heterologous polySTs were expressed in trans and the linkages of the resulting PSA products determined by a simple immunological screen, verified independently by NMR structural analysis. The results identified the N-terminal domains of each polyST as salient to linkage specificity, thus supporting a two-site model of catalysis. Site-directed mutagenesis further characterized specificity and identified catalytically important amino acids among the nine conserved aspartate or glutamate residues, which include the catalytic base and residues coordinating donor (CMP-sialic acid) binding. These results provide a valuable experimental scaffold for understanding the biosynthesis of the bacterial PSA virulence factor.

L6 ANSWER 6 OF 18 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation on STN DUPLICATE 2

ACCESSION NUMBER: 2001:646142 SCISEARCH
THE GENUINE ARTICLE: 461DH
TITLE: Elongation of alternating alpha 2,8/2,9 polysialic acid by the Escherichia coli **K92** **polysialyltransferase**
AUTHOR: McGowen M M; Vionnet J; Vann W F (Reprint)
CORPORATE SOURCE: Ctr Biol Evaluat & Res, Lab Bacterial Toxins, Div Bacterial Parasit & Allergen Prod, 8800 Rockville Pike, Bethesda, MD 20892 USA (Reprint); Ctr Biol Evaluat & Res, Lab Bacterial Toxins, Div Bacterial Parasit & Allergen Prod, Bethesda, MD 20892 USA
COUNTRY OF AUTHOR: USA
SOURCE: GLYCOBIOLOGY, (AUG 2001) Vol. 11, No. 8, pp. 613-620. ISSN: 0959-6658.
PUBLISHER: OXFORD UNIV PRESS INC, JOURNALS DEPT, 2001 EVANS RD, CARY, NC 27513 USA.
DOCUMENT TYPE: Article; Journal
LANGUAGE: English
REFERENCE COUNT: 21
ENTRY DATE: Entered STN: 24 Aug 2001
Last Updated on STN: 24 Aug 2001

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB We have chosen E. coli **K92**, which produces the alternating structure alpha (2-8)neuNAc alpha (2-9)neuNAc as a model system for studying bacterial polysaccharide biosynthesis. We have shown that the **polysialyltransferase** encoded by the **K92** neuS gene can synthesize both alpha (2-8) and alpha (2-9) neuNAc linkages in vivo by C-13-nuclear magnetic resonance analysis of polysaccharide isolated from a heterologous strain containing the **K92** neuS gene. The **K92 polysialyltransferase** is associated with the membrane in lysates of cells harboring the neuS gene in expression vectors. Although the enzyme can transfer sialic acid to the nonreducing end of oligosaccharides with either linkage, it is unable to initiate chain synthesis without exogenously added polysialic acid. Thus, the **polysialyltransferase** encoded by neuS is not sufficient for de novo synthesis of polysaccharide but requires another membrane component for initiation. The acceptor specificity of this **polysialyltransferase** was studied using sialic acid oligosaccharides of various structures as exogenous acceptors. The enzyme can transfer to the nonreducing end of all bacteria polysialic acids, but has a definite preference for alpha (2-8) acceptors. Gangliosides containing neuNAc oc(2-8)neuNAc are elongated, whereas monsialylated gangliosides are not. Disialylgangliosides are better acceptors than short oligosaccharides, suggesting a lipid-linked oligosaccharide may be preferred in the elongation reaction. These studies show that the **K92 polysialyltransferase** catalyzes an elongation

reaction that involves transfer of sialic acid from CMP-sialic acid to the nonreducing end of two different acceptor substrates.

L6 ANSWER 7 OF 18 CAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 2000:133873 CAPLUS
DOCUMENT NUMBER: 132:162041
TITLE: Escherichia coli strain **K92** gene neuS α2,8/2,9- **polysialyltransferase**, its recombinant production, purification and activity
INVENTOR(S): Wong, Chi-Huey; Shen, Gwo-Jenn; Datta, Arun
PATENT ASSIGNEE(S): Scripps Research Institute, USA
SOURCE: PCT Int. Appl., 33 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2000009736	A1	20000224	WO 1999-US18154	19990810
W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
CA 2339173	AA	20000224	CA 1999-2339173	19990810
AU 9954758	A1	20000306	AU 1999-54758	19990810
EP 1105515	A1	20010613	EP 1999-941028	19990810
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO				

PRIORITY APPLN. INFO.: US 1998-96003P P 19980810
WO 1999-US18154 W 19990810

AB The invention presents the recombinant production of gene neuS α2,8/2,9- **polysialyltransferase** from Escherichia coli **K92**. The invention provides plasmid vectors containing the neuS gene, host cells transformed with said vectors, and processes involved in obtaining the purified recombinant α2,8/2,9- **polysialyltransferase**. The invention also provides methods used to show that E. coli **K92** α2,8/2,9- **polysialyltransferase** is a functional enzyme able to convert a substrate to product. The invention specifically demonstrated PCR-based cloning of the E. coli neuS gene. A 1.2-kb PCR fragment was subcloned in pRSET vector and the protein was expressed in the BL21(DE3) strain of E. coli with a hexameric histidine at its N-terminal end. Western blotting using anti-histidine antibody showed the presence of a band that migrated at about 47.5 kD on both reducing and non-reducing SDS-PAGE, indicating a monomeric enzyme. Among the carbohydrate acceptors tested, N-acetylneuraminic acid and the gangliosides GD3 and GQ1b were preferred substrates. The cell-free enzyme reaction products obtained were characterized by NMR and mass spectrometry, which indicated the presence of both α2,9- and α2,8-linked polysialyl structure. The **K92** neuS gene was used to transform the K1 strain of E. coli, the capsule of which contains only (-8-NeuAcα2-) linkages. Anal. of the polysaccharides isolated from these transformed cells is consistent with the presence of both (-8-NeuAcα2-) and (-9-NeuAcα2-) linkages, thus supporting that E. coli **K92** catalyzes the synthesis of polysialic acid with α2,9- and α2,8- linkages.

REFERENCE COUNT: 3 THERE ARE 3 CITED REFERENCES AVAILABLE FOR THIS

RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L6 ANSWER 8 OF 18 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation on STN DUPLICATE 3

ACCESSION NUMBER: 1999:930674 SCISEARCH
THE GENUINE ARTICLE: 260XW
TITLE: Expression of alpha 2,8/2,9-**polysialyltransferase** from Escherichia coli **K92** - Characterization of the enzyme and its reaction products
AUTHOR: Shen G J; Datta A K; Izumi M; Koeller K M; Wong C H (Reprint)
CORPORATE SOURCE: Scripps Res Inst, Dept Chem, 10666 N Torrey Pines Rd, BCC 338, San Diego, CA 92037 USA (Reprint); Scripps Res Inst, Dept Chem, La Jolla, CA 92037 USA; Scripps Res Inst, Skaggs Inst Chem Biol, La Jolla, CA 92037 USA
COUNTRY OF AUTHOR: USA
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, (3 DEC 1999) Vol. 274, No. 49, pp. 35139-35146.
ISSN: 0021-9258.
PUBLISHER: AMER SOC BIOCHEMISTRY MOLECULAR BIOLOGY INC, 9650 ROCKVILLE PIKE, BETHESDA, MD 20814 USA.
DOCUMENT TYPE: Article; Journal
LANGUAGE: English
REFERENCE COUNT: 41
ENTRY DATE: Entered STN: 1999
Last Updated on STN: 1999

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB The capsular polysaccharide of Escherichia coil **K92** contains alternating -8-NeuAc alpha 2- and -9-NeuAc alpha 2- linkages. The enzyme catalyzing this polymerizing reaction has been cloned from the genomic DNA of E. coil **K92**. The 1.2-kilobase polymerase chain reaction fragment was subcloned in pRSET vector and the protein was expressed in the BL21(DE3) strain of E. coli with a hexameric histidine at its N-terminal end, The enzyme was isolated in the supernatant after lysis of the cells and fractionated by ultracentrifugation. Western blotting using anti-histidine antibody showed the presence of a band that migrated at about 47.5 kDa on both reducing and nonreducing SDS-polyacrylamide gel electrophoresis, indicating a monomeric enzyme. Among the carbohydrate accepters tested, N-acetylneuraminic acid and the gangliosides G(D3) and G(Q1b) were preferred substrates. The cell-free enzyme reaction products obtained were characterized by NMR and mass spectrometry, which indicated the presence of both alpha 2,9- and alpha 2,8-linked polysialyl structure. The **K92** neuS gene was used to transform the K1 strain of E. coil, the capsule of which contains only -8-NeuAc alpha 2- linkages. Analysis of the polysaccharides isolated from these transformed cells is consistent with the presence of both -8-NeuAc alpha 2- and -9-NeuAc alpha 2- linkages. Our results suggest that the neuS gene product of E. coil **K92** catalyzes the synthesis of polysialic acid with alpha 2,9- and alpha 2,8-linkages in vitro and in vivo.

L6 ANSWER 9 OF 18 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation on STN

ACCESSION NUMBER: 1999:477906 SCISEARCH
THE GENUINE ARTICLE: 209PJ
TITLE: On the biosynthesis of alternating alpha-2,9/alpha-2,8 heteropolymer of sialic acid catalyzed by the sialyltransferase of Escherichia coli Bos-12
AUTHOR: Chao C F; Chuang H C; Chiou S T; Liu T Y (Reprint)
CORPORATE SOURCE: Acad Sinica, Inst Biol Chem, Taipei 115, Taiwan (Reprint)
COUNTRY OF AUTHOR: Taiwan
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, (25 JUN 1999) Vol. 274, No. 26, pp. 18206-18212.
ISSN: 0021-9258.

PUBLISHER: AMER SOC BIOCHEMISTRY MOLECULAR BIOLOGY INC, 9650 ROCKVILLE PIKE, BETHESDA, MD 20814 USA.
DOCUMENT TYPE: Article; Journal
LANGUAGE: English
REFERENCE COUNT: 21
ENTRY DATE: Entered STN: 1999
Last Updated on STN: 1999
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Escherichia coli Bos-la synthesizes a heteropolymer of sialic acids with alternating alpha-2,9/alpha-2,8 glycosidic linkages (1), In this study, we have shown that the **polysialyltransferase** of the E. coli Bos-12 recognizes an a-2,8 glycosidic linkage of sialic acid at the nonreducing end of an exogenous acceptor of either the alpha-2,8 homopolymer of sialic acid or the alternating alpha-2,9/alpha-2,8 heteropolymer of sialic acid and catalyzes the transfer of Neu5Ac from CMP-Neu5Ac to this residue. When the exogenous acceptor is an alpha-a,8-linked oligomer of sialic acid, the main product synthesized is derived from the addition of a single residue of [C-14]Neu5Ac to form either an alpha-2,8 glycosidic linkage or an alpha-2,9 glycosidic linkage at the nonreducing end, at an alpha-2,8/alpha-2,9 ratio of approximately 2:1, When the acceptor is the alternating alpha-2,9/alpha-2,8 heteropolymer of sialic acid, chain elongation takes place four to five times more efficiently than the alpha-2,8-linked homopolymer of sialic acid as an acceptor. It was found that the alpha-2,9-linked homopolymer of sialic acid and the alpha-2,8/alpha-2,9-linked hetero-oligomer of sialic acid with alpha-2,9 at the nonreducing end not only failed to serve as an acceptor for the E. coli Bos-la **polysialyltransferase** for the transfer of [C-14]Neu5Ac, but they inhibited the de novo synthesis of polysialic acid catalyzed by this enzyme. The results obtained in this study favor the proposal that the biosynthesis of the alpha-2,9/alpha-2,8 heteropolymer of sialic acid catalyzed by the E. coil Bos-la **polysialyltransferase** involves a successive transfer of a preformed alpha-2,8-linked dimer of sialic acid at the nonreducing terminus of the acceptor to form an alpha-2,9 glycosidic linkage between the incoming dimer and the acceptor. The glycosidic linkage at the nonreducing end of the alternating alpha-2,9/alpha-2,8 heteropolymer of sialic acid produced by E, coli Bos-la should be an alpha-2,8 glycosidic bond and not an alpha-2,9 glycosidic linkage.

L6 ANSWER 10 OF 18 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation on STN DUPLICATE 4
ACCESSION NUMBER: 1999:144307 SCISEARCH
THE GENUINE ARTICLE: 166KQ
TITLE: Haemophilus ducreyi produces a novel sialyltransferase - Identification of the sialyltransferase gene and construction of mutants deficient in the production of the sialic acid-containing glycoform of the lipooligosaccharide
AUTHOR: Bozue J A; Tullius M V; Wang J; Gibson B W; Munson R S (Reprint)
CORPORATE SOURCE: Childrens Hosp Res Fdn, 700 Childrens Dr, Room W402, Columbus, OH 43205 USA (Reprint); Childrens Hosp Res Fdn, Columbus, OH 43205 USA; Ohio State Univ, Dept Pediat, Columbus, OH 43205 USA; Ohio State Univ, Dept Med Microbiol, Columbus, OH 43205 USA; Univ Calif San Francisco, Dept Pharmaceut Chem, San Francisco, CA 94143 USA
COUNTRY OF AUTHOR: USA
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, (12 FEB 1999) Vol. 274, No. 7, pp. 4106-4114.
ISSN: 0021-9258.
PUBLISHER: AMER SOC BIOCHEMISTRY MOLECULAR BIOLOGY INC, 9650 ROCKVILLE PIKE, BETHESDA, MD 20814 USA.

DOCUMENT TYPE: Article; Journal
LANGUAGE: English
REFERENCE COUNT: 76
ENTRY DATE: Entered STN: 1999
Last Updated on STN: 1999
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Haemophilus ducreyi, the cause of the sexually transmitted disease chancroid produces a lipooligosaccharide (LOS) containing a terminal sialyl N-acetyllactosamine trisaccharide, Previously, we reported the identification and characterization of the N-acetylneuraminic acid cytidylsynthetase gene (neuA), Forty-nine base pairs downstream of the synthetase gene is an open reading frame (ORF) encoding a protein with a predicted molecular weight of 34,646, This protein has weak homology to the **polysialyltransferase** of Escherichia colt **K92**, Downstream of this ORF is the gene encoding the H, ducreyi homologue of the Salmonella typhimurium rmlB gene. Mutations were constructed in the neuA gene and the gene encoding the second ORF by insertion of an Omega kanamycin cassette, and isogenic strains were constructed. LOS was isolated from each strain and characterized by SDS-polyacrylamide gel electrophoresis, carbohydrate, and mass spectrometric analysis, LOS isolated from strains containing a mutation in neuA or in the second ORF, designated Ist, lacked the sialic acid-containing glycoform, Complementation studies were performed, The neuA gene and the ist gene were each cloned into the shuttle vector pLS88 after polymerase chain reaction amplification. Complementation of the mutation in the ist gene was observed, but we were unable to complement the neuA mutation, Since it is possible that transcription of the neuA gene and the Ist gene were coupled, we constructed a nonpolar mutation in the neuA gene, in this construct, the neuA mutation was complemented, suggesting transcriptional coupling of the neuA gene and the ist gene, Sialyltransferase activity was detected by incorporation of C-14-labeled NeuAc from CMP-NeuAc into trichloroacetic acid-precipitable material when the Ist gene was overexpressed in the nonpolar neuA mutant. We conclude that the Ist gene encodes the H, ducreyi sialyltransferase, Since the Ist gene product has little, if any, structural relationship to other sialyltransferases, this protein represents a new class of sialyltransferase.

L6 ANSWER 11 OF 18 CAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 1999:539790 CAPLUS
TITLE: Biosynthesis of polysialic acid as capsular polysaccharides in .
AUTHOR(S): McGowen, Margaret M.; Vionnet, Justine A.; Scates, Bradley A.; Vann, Willie F.
CORPORATE SOURCE: Center for Biologics Evaluation and Research, Bethesda, MD, 20892, USA
SOURCE: Book of Abstracts, 218th ACS National Meeting, New Orleans, Aug. 22-26 (1999), CARB-056. American Chemical Society: Washington, D. C.
CODEN: 67ZJA5
DOCUMENT TYPE: Conference; Meeting Abstract
LANGUAGE: English

AB Polysialic acids are synthesized by pathogenic bacteria as capsular polysaccharides. These polymers have been implicated in the virulence of some strains of Escherichia coli which cause neonatal meningitis and urinary tract infections. There has been significant progress in identifying the gene necessary for capsular polysacccharide biosynthesis in gram neg. bacteria. Much of the enzymol. of polysialic acid capsular polysaccharide synthesis has been done with the a-(2-8) **polysialyltransferase** complex of E.coli K1. Bacteria containing DNA fragments encoding several capsule related genes have been used as a source of enzyme activity. As a model system for investigating the mechanism of capsular glycosyltransferases we have chosen to investigate the **K92** a(2-8)(2-9)**polysialyltransferase** in a genetic

background lacking other capsule related genes. The **K92** PST requires an exogenously added acceptor when assayed in this genetic background. The **K92 polysialyltransferase** does not require neuE gene product for activity. The effect of acceptor repeat unit structure, chain lenght on elongation activity was determined by the addition of other sialic acid polymers, oligosialic acid, and gangliosides.

L6 ANSWER 12 OF 18 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation on STN

ACCESSION NUMBER: 1998:793561 SCISEARCH
THE GENUINE ARTICLE: 130CC
TITLE: Expression of the Escherichia coli **K92 polysialyltransferase**
AUTHOR: Vionnet J A (Reprint); McGowen M M; Scates B A; Vann W F
CORPORATE SOURCE: US FDA, Ctr Biol Evaluat & Res, Bethesda, MD USA
COUNTRY OF AUTHOR: USA
SOURCE: GLYCOBIOLOGY, (NOV 1998) Vol. 8, No. 11, pp. 1124-1125. MA 79.
ISSN: 0959-6658.
PUBLISHER: OXFORD UNIV PRESS INC, JOURNALS DEPT, 2001 EVANS RD, CARY, NC 27513 USA.
DOCUMENT TYPE: Conference; Journal
LANGUAGE: English
REFERENCE COUNT: 0
ENTRY DATE: Entered STN: 1998
Last Updated on STN: 1998

L6 ANSWER 13 OF 18 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation on STN DUPLICATE 5

ACCESSION NUMBER: 1995:327642 SCISEARCH
THE GENUINE ARTICLE: QX543
TITLE: A FILTER ASSAY FOR **POLYSIALYLTRANSFERASE**
AUTHOR: VANN W F (Reprint)
CORPORATE SOURCE: US FDA, CTR BIOL EVALUAT & RES, BACTERIAL POLYSACCHARIDES LAB, 8800 ROCKVILLE PIKE, BETHESDA, MD 20892 (Reprint)
COUNTRY OF AUTHOR: USA
SOURCE: FEMS MICROBIOLOGY LETTERS, (1 MAY 1995) Vol. 128, No. 2, pp. 163-166.
ISSN: 0378-1097.
PUBLISHER: ELSEVIER SCIENCE BV, PO BOX 211, 1000 AE AMSTERDAM, NETHERLANDS.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE
LANGUAGE: English
REFERENCE COUNT: 19
ENTRY DATE: Entered STN: 1995
Last Updated on STN: 1995

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Polysialic acids occur as capsular polysaccharides of several pathogenic bacteria. An understanding of how **polysialyltransferase** functions in the synthesis of polysialic acid will require enzyme purification and characterization in concert with genetic analysis. A rapid filter assay has been developed for bacterial **polysialyltransferase** suitable for enzyme purification. The filter assay and the currently used paper chromatography methods are equivalent in parallel experiments. The Escherichia coli **K92 polysialyltransferase** exhibited the same pH and temperature optima, Mg2+ dependence and acceptor specificity in both assays. [C-14]Sialic acid bound in filter assays correlates with polymer formed by gel filtration. Specificity may be increased by the addition of exogenous accepters.

L6 ANSWER 14 OF 18 CAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 1993:577338 CAPLUS
DOCUMENT NUMBER: 119:177338
TITLE: Mechanisms of polysialic acid assembly in Escherichia coli K1: A paradigm for microbes and mammals
AUTHOR(S): Vimr, Eric R.; Steenbergen, Susan M.
CORPORATE SOURCE: Coll. Vet. Med., Univ. Illinois, Urbana, IL, 61801, USA
SOURCE: Polysialic Acid (1993), 73-91. Editor(s): Roth, Juergen; Rutishauser, Urs; Troy, Frederick A., II. Birkhaeuser: Basel, Switz.
CODEN: 59FNAM
DOCUMENT TYPE: Conference
LANGUAGE: English

AB A genetic system was developed to investigate the mol. mechanisms of α2,8-linked polysialic acid (PSA) capsule synthesis, translocation, and regulation in the neuroinvasive bacterium E. coli K1. The 12 to 14 genes required for these processes are located in a multigenic kps cluster at chromosome unit 64. The cluster is composed of a central group of biosynthetic neu genes (region 2) that are flanked on either side by region 1 or 3 kps genes encoding general functions for PSA regulation, assembly, and translocation. The **polysialyltransferase** (polyST) encoded by K1 neuS was sequenced and compared to its homolog in **K92** E. coli, which synthesizes PSA chains with alternating sialyl α2,8-2,9 linkages. The results indicate that polySTs are processive enzymes which catalyze sequential transsialylations from donor CMP-sialic acid mols. to the nonreducing end of nascent PSA chains. The authors propose that the polymerase functions in a complex that includes the region 2 gene product of neuE and region 1 and 3 gene products of kpsMTSCDE. NeuE appears to function in the initiation or termination of PSA synthesis and may interact with polyprenol, as suggested by a dolichol-like binding site located in its predicted C-terminal membrane-spanning domain. These conclusions are supported by phenotypic anal. of mutants with multiple defects in sialic acid synthesis, degradation, and polymerization

L6 ANSWER 15 OF 18 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation on STN DUPLICATE 6
ACCESSION NUMBER: 1992:457244 SCISEARCH
THE GENUINE ARTICLE: JF345
TITLE: HOMOLOGY AMONG ESCHERICHIA-COLI K1 AND **K92** **POLYSIALYLTRANSFERASES**
AUTHOR: VIMR E R (Reprint); BERGSTROM R; STEENBERGEN S M; BOULNOIS G; ROBERTS I
CORPORATE SOURCE: UNIV ILLINOIS, COLL VET MED, DEPT PATHOBIOL, URBANA, IL 61801 (Reprint); UNIV LEICESTER, DEPT MICROBIOL, LEICESTER LE1 9HN, ENGLAND
COUNTRY OF AUTHOR: USA; ENGLAND
SOURCE: JOURNAL OF BACTERIOLOGY, (AUG 1992) Vol. 174, No. 15, pp. 5127-5131.
ISSN: 0021-9193.
PUBLISHER: AMER SOC MICROBIOLOGY, 1325 MASSACHUSETTS AVENUE, NW, WASHINGTON, DC 20005-4171.
DOCUMENT TYPE: Note; Journal
FILE SEGMENT: LIFE
LANGUAGE: English
REFERENCE COUNT: 31
ENTRY DATE: Entered STN: 1994
Last Updated on STN: 1994
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB The neuS-encoded **polysialyltransferase** (polyST) in Escherichia coli K1 catalyzes synthesis of polysialic acid homopolymers composed of unbranched sialyl-alpha-2,8 linkages. Subcloning and

complementation experiments showed that the K1 neuS was functionally interchangeable with the neuS from E. coli **K92** (S. M. Steenbergen, T. J. Wrona, and E. R. Vimr, J. Bacteriol. 174:1099-1108, 1992), which synthesizes polysialic acid capsules with alternating sialyl-alpha-2,8-2,9 linkages. To better understand the relationship between these polySTs, the complete **K92** neuS sequence was determined. The results demonstrated that K1 and **K92** neuS genes are homologous and indicated that the **K92** copy may have evolved from its K1 homolog. Both K1 and **K92** structural genes comprised 1,227 bp. There were 156 (12.7%) differences between the two sequences; among these mutations, 55 did not affect the derived primary structure of **K92** polyST and hence were synonymous with the K1 sequence. Assuming maximum parsimony, another estimated 17 synonymous mutations plus 84 nonsynonymous mutations could account for the 70 amino acid replacements in **K92** polyST; 36 of these replacements were judged to be conservative when compared with those of K1 polyST. There were no changes detected in the first 146 5' or last 129 3' bp of either gene, suggesting, in addition to the observed mutational differences, the possibility of a past recombination event between neuS loci of two different kps clusters. The results indicate that relatively few amino acid changes can account for the evolution of a glycosyltransferase with novel linkage specificity.

L6 ANSWER 16 OF 18 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation on STN DUPLICATE 7

ACCESSION NUMBER: 1992:113388 SCISEARCH
THE GENUINE ARTICLE: HD467
TITLE: FUNCTIONAL-ANALYSIS OF THE SIALYLTRANSFERASE COMPLEXES IN ESCHERICHIA-COLI K1 AND **K92**
AUTHOR: STEENBERGEN S M (Reprint); WRONA T J; VIMR E R
CORPORATE SOURCE: UNIV ILLINOIS, COLL VET MED, DEPT PATHOBIOL, URBANA, IL 61801
COUNTRY OF AUTHOR: USA
SOURCE: JOURNAL OF BACTERIOLOGY, (FEB 1992) Vol. 174, No. 4, pp. 1099-1108.
ISSN: 0021-9193.
PUBLISHER: AMER SOC MICROBIOLOGY, 1325 MASSACHUSETTS AVENUE, NW, WASHINGTON, DC 20005-4171.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE
LANGUAGE: English
REFERENCE COUNT: 38
ENTRY DATE: Entered STN: 1994
Last Updated on STN: 1994

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB The **polysialyltransferase** (polyST) structural gene, neuS, for poly-alpha-2,8sialic acid (PSA) capsule synthesis in Escherichia coli K1 was previously mapped near the kps region 1 and 2 junction (S. M. Steenbergen and E. R. Vimr, Mol. Microbiol. 4:603-611, 1990). Present Southern and colony blot hybrization results confirmed that neuS was a region 2 locus and indicated apparent homology with neuS from E. coli **K92**, bacteria that synthesize a sialyl-alpha-2,8-2,9-linked polymer. A K1- mutant with an insertion mutation in neuS was complemented in trans by **K92** neuS, providing direct evidence that neuS encoded the PSA polymerase. A 2.9-kb E. coli K1 kps subclone was sequenced to better characterize polyST. In addition to neuS, the results identified a new open reading frame, designated neuE, the linker sequence between regions 1 and 2, and the last gene of region 1, kpsS. The kpsS translational reading frame was confirmed by sequencing across the junction of a kpsS'-lacZ+ fusion. PolyST was identified by maxicell analysis of nested deletions and coupled in vitro transcription-translation assays. PolyST's derived primary structure predicted a 47,500-Da basic polypeptide without extensive similarity to other known

proteins. PolyST activity was increased 31-fold and was membrane localized when neuS was cloned into an inducible expression vector, suggesting, together with the polyST primary structure, that polyST is a peripheral inner membrane glycosyltransferase. However, polyST could not initiate de novo PSA synthesis, indicating a functional requirement for other kps gene products. The existence of a sialyltransferase distinct from polyST was suggested by identification of a potential polyprenyl-binding motif in a C-terminal membrane-spanning domain of the predicted neuE gene product. Direct evidence for a quantitatively minor sialyltransferase activity, which could function to initiate PSA synthesis, was obtained by phenotypic analysis of mutants with multiple defects in sialic acid synthesis, degradation, and polymerization. The results provide an initial molecular description of K1 and **K92** sialyltransferase complexes and suggest a possible common function for accessory kps gene products.

L6 ANSWER 17 OF 18 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN

ACCESSION NUMBER: 1992:380977 BIOSIS
DOCUMENT NUMBER: PREV199243047927; BR43:47927
TITLE: SEQUENCE AND STRUCTURAL HOMOLOGY BETWEEN ESCHERICHIA-COLI K1 AND **K92 POLYSIALYLTRANSFERASES**.
AUTHOR(S): STEENBERGEN S [Reprint author]; BERGSTROM R; VIMR E
CORPORATE SOURCE: UNIV ILL, URBANA, ILL, USA
SOURCE: Abstracts of the General Meeting of the American Society for Microbiology, (1992) Vol. 92, pp. 134.
Meeting Info.: 92ND GENERAL MEETING OF THE AMERICAN SOCIETY FOR MICROBIOLOGY, NEW ORLEANS, LOUISIANA, USA, MAY 26-30, 1992. ABSTR GEN MEET AM SOC MICROBIOL.
ISSN: 1060-2011.
DOCUMENT TYPE: Conference; (Meeting)
FILE SEGMENT: BR
LANGUAGE: ENGLISH
ENTRY DATE: Entered STN: 17 Aug 1992
Last Updated on STN: 17 Aug 1992

L6 ANSWER 18 OF 18 TOXCENTER COPYRIGHT 2005 ACS on STN

ACCESSION NUMBER: 2004:323370 TOXCENTER
DOCUMENT NUMBER: CRISP-2003-BJ004013-01
TITLE: Glycobiology of Bacterial Pathogens
AUTHOR(S): VANN W F
SUPPORTING ORGANIZATION (SPONSORING AGENCY): U.S. DEPT. OF HEALTH AND HUMAN SERVICES; PUBLIC HEALTH SERVICE; NATIONAL INSTITUTES OF HEALTH, CENTER FOR BIOLOGICS EVALUATION AND RESEARCH - BACTRIAL PRODUCTS
SOURCE: Crisp Data Base National Institutes of Health.
DOCUMENT TYPE: (Research)
FILE SEGMENT: CRISP
LANGUAGE: English
ENTRY DATE: Entered STN: 20041229
Last Updated on STN: 20041229

AB Bacterial pathogens are often coated with polysaccharide virulence factors or produce toxins that bind to host oligosaccharides as receptors for cells entry. These polysaccharides and toxins continue to be excellent targets for prevention and controlling disease. Indeed inactivated toxins, polysaccharides, and polysaccharides conjugated to inactivated toxins form the basis for several of the currently licensed and future vaccines. The goals of this research program are to determine(a)the metabolic pathway for the synthesis of polysialic acids in gram negative pathogens and (b) the interaction of neurotoxins of Clostridium botulinum and C. tetani with the glycolipids found on nerve cells. As a part of the the counterterrorism effort at CBER we are also (c)developing an assay to determine antibodies to botulinum toxin and (d)characterizing the

hyaluronic acid biosynthetic genes in Bacillus anthracis. Structure and function of enzymes involved the metabolism of polysialic acid. Polysialic acids are synthesized by pathogenic bacteria as capsular polysaccharides. These polymers have been implicated in the virulence of some strains of Escherichia coli, which cause neonatal meningitis and urinary tract infections. Polysialic acid is also present in the developing human brain. There has been significant progress in identifying the gene necessary for capsular polysaccharide biosynthesis in gram negative bacteria. The objective of this project is to determine the mechanism of capsular polysaccharide biosynthesis in virulent encapulated bacteria. Our approach is to characterize the structure and function of the enzymes in the pathway and to use them as tools for understanding sialylation in bacteria and humans. Much of the enzymology of polysialic acid capsular polysaccharide synthesis has been done with the a(2-8) **polysialyltransferase** complex of E. coli K1. Bacteria containing DNA fragments encoding several capsule related genes have been used as a source of enzyme activity. As a model system for investigating the mechanism of capsular glycosyltransferases we have chosen to investigate the **K92** a(2-8)(2-9) **polysialyltransferase** in a genetic background lacking other capsule related genes. The neuS gene encodes this glycosyltransferase and is the only glycosyltransferase to date identified with synthesis of this polymer. We have shown that the **K92** neuS gene product can synthesize both a(2,8) and a(2,9) neuNAc linkages in vitro in a background free of other capsule related gene products and confirmed in vivo synthesis of this polymer by 13C-NMR. We have shown that the acceptor specificity for polysialic acids is broad for thisenzyme. Gangliosides containing this disialylated oligosaccharide are elongated, while monsialylated gangliosides are not. Disialylgangliosides are better acceptors than short oligosaccharides suggesting a role for lipid in the elongation reaction. The idea of a lipid linked acceptor is further supported by the elongation of a disialyloligosaccharide possessing a hydrophobic aglycon. We have extracted membranes of strains defective in sialic acid and therefore polysaccharide synthesis and demonstrated the presence of an acceptor in these extracts. The acceptor is not a protein and behaves chromatographically like a glycolipid. This acceptor will support the formation of polymer by the **K92** neuS **polysialyltransferase**. The lipid does not behave like an undecaprenol. Sialic acid is synthesized from N-acetylmannosamine and phosphoenol pyruvate. We have determine the sterochemistry of this condensation reaction. We have also shown that the source of the N-acetylmannosamine in the pathway is UDP-N-acetylglucosamine. The gene that encodes the enzyme catalyzing this conversion is neuC.
Characterization of tetanus toxin structure and function. Clostridial neurotoxins (botulinum and tetanus toxins) are large proteins organized into three functional domains, an amino terminal proteolytic domain, central translocation domain, and a carboxyl terminal receptor binding region. The neurotoxic effects of clostridial neurotoxins results from binding to cell surface receptors, translocation into the neural cell, and the proteolytic cleavage of proteins essential for synaptic vesicle docking/fusion events (SNARE proteins) by the enzymatically active amino terminal domain. The subsequent block of neurotransmitter release at the neuromuscular junction by botulinum toxins or block of inhibitory neurotransmitter release within the central nervous system by tetanus toxin leads to flaccid or spasmodic paralysis of the victim, respectively. The focus of this project has been the characterization of the receptor binding domain of tetanus C-fragment. To determine which amino acids in tetanus toxin are involved in ganglioside binding, homology modeling was performed using recently resolved X-ray crystallographic structures of tetanus toxin HC fragment. Based on these analyses, two regions in tetanus toxin that share structural homology with the binding domains of other sialic acid and galactose-binding proteins were targeted for mutagenesis. Specific amino acids within these regions were altered using site-directed mutagenesis. The amino acid residue tryptophan-1288 was

found to be critical for binding of the HC fragment to ganglioside GT1b. Docking of GD1b within this region of the toxin suggested that histidine-1270 and aspartate-1221 were within hydrogen bonding distance of the ganglioside. These two residues were mutagenized and found to also be important for the binding of tetanus toxin HC fragment to ganglioside GT1b. In addition, the HC fragments mutagenized at these residues have reduced levels of binding to neurites of differentiated PC-12 cells. These studies indicate that the amino acids tryptophan-1288, histidine-1270, and aspartate-1221 are components of the GT1b-binding site on the tetanus toxin HC fragment. Biosynthesis of polysaccharides in Bacillus anthracis. Bacillus anthracis is a gram-positive bacterium that causes Anthrax. Two megaplasmids pXO1 and pXO2 in B. anthracis have been shown to encode for the virulence factors of this bacteria. Biosynthesis of the anthrax toxin requires the presence of the pXO1 plasmid (110 Mda), since it contains the genes encoding for the proteins protective antigen, lethal factor and edema factor. The other plasmid pXO2 (60 Mda) contains the genes capB, capC, capA and dep that are required for the synthesis of poly-D-glutamic acid capsule. Both of these plasmids are required for the full virulence of B. anthracis. In spite of the importance of these two plasmids for the virulence, many of the genes in these plasmids are yet to be studied in detail. Recently, the sequence and organization of the plasmid pXO1 has been reported by Okinaka et al and has been shown to contain a total of 143 ORFs. Functions for the proteins encoded by 35 of these ORFs have been assigned based on their similarities to proteins from other organisms. All the toxin genes in the pXO1 plasmid are contained in a 44.8-kb region designated as pathogenecity island (PAI). Interestingly, ORFs 93, 94 and 95 which encode for proteins with sequence similarity to hyaluronic acid synthetase (hasA), UDP-glucose-pyrophosphorylase (hasC) and UDP-glucose-dehydrogenase (hasB) of Streptococcus are present immediately adjacent to the PAI. Hyaluronic acid is capsular polysaccharide and a virulence factor for Streptococcus pyogenes and Pasteurella multocida. There is no precedence for the presence of a polysaccharide capsule in B. anthracis. Therefore, it is not known if these genes (ORFs 93, 94 and 95) are even functional and even if they express to form the corresponding proteins, their importance in terms of virulence is unknown. We have amplified and express 2 of the putative hyaluronic acid synthesis genes. Western blot analysis of the culture lysates of these E. coli cells indicated that both the UDP-glucose dehydrogenase and the hyaluronic acid synthase were expressed in this system. Recombinant B. anthracis hyaluronic synthase did not appear to catalyze the formation of hyaluronic acid in vitro. Closer examination f the sequence of this gene cluster revealed a frame shift mutation. We corrected this frame shift by site directed mutagenesis. The mutated protein product obtained from these E. coli cells was assayed for their ability to form hyaluronic acid. We find that this large protein incorporates glcNAc into high MW material. We are currently studying the biochemical characteristics of this mutant protein as well as the polysaccharide that is formed by this protein. Our preliminary studies with UDP-glucose dehydrogenase showed that this enzyme is active and that it can utilize both UDP-glucose . Determination of Antibody of Botulinum toxins. Assay development for antibody against botulinum toxin will contribute to effective testing of regulated products by developing in vitro tests of toxin presence and potency. Current assays for botulinum toxin are based on mouse lethality and are therefore lengthy, expensive and imprecise. Most neutralizing antibody induced by botulinum toxoid vaccine is against the receptor binding domain. We propose to develop a convenient and sensitive method to measure this type of antibody. We have reinvestigated the parameters used for measuring the binding of tetanus Hc fragment to GT1b coated Biacore chip and determine binding constants for tetanus Hc to GT1b. We have constructed chimeras of tetanus Hc and indicator fragments that will be adaptable to ELISA assays. We have prepared derivatized GT1b oligosaccharides to facilitate coating to microtiter plates. This project incorporates FY2002 projects

1Z01BJ004001-12, 1Z01BJ004005-08, 1Z01BJ004006-02, and 1Z01BJ004010-01.